Molecular Biology

A SELEX PROCEDURE FOR DETERMINING OPTIMAL DNA BINDING SITES OF THE *Pseudomonas aeruginosa* REGULATORY PROTEIN AlgZ

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Pseudomonas aeruginosa is a gram-negative bacterium found in soil and water. It is an opportunistic pathogen in patients with the genetic disorder cystic fibrosis (CF). *P. aeruginosa* initially colonizes the lungs of CF patients with nonmucoid strains, but these strains later mutate, secrete the exopolysaccharide alginate, and express a mucoid (slimy) phenotype. Isolation of mucoid colonies from the CF lung is associated with a poor prognosis for the patient because alginate provides resistance to the human immune system.

Transcriptional activation of the *P. aeruginosa* alginate biosynthetic operon results in the synthesis of alginate. *algD* is the first gene of this operon and encodes GDP-mannose dehydrogenase, an enzyme that commits precursor molecules to the production of alginate. The regulatory protein AlgZ is crucial for alginate production as both an activator of *algD* transcription and also a repressor of its own synthesis. The sequences of the two known binding sites of AlgZ to the genome of *P. aeruginosa* differ greatly, although a centralized motif has been hypothesized. A dSELEX (systematic evolution of ligands by exponential enrichment with degeneracy) procedure was utilized to isolate AlgZ targets from a random pool of DNA fragments. dSELEX is a process that allows pieces of DNA that bind with high-affinity to a protein to dominate a random DNA pool. The isolated DNA targets were cloned into a plasmid, and transformed into *E. coli* cells. Plasmids containing an AlgZ binding insert were isolated from successful clones. These clones will be examined via sequence analysis and subsequent sequence alignment should reveal any AlgZ-binding motif(s).